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Membrane Active Peptides and Toxins II

2771-Pos Board B201
The Presence of Antiparallel Beta-Sheets in Toxic Fibrils Formed by ABeta on GM1 Clusters
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The abnormal aggregation of amyloid β-protein (Aβ) is considered to be central in the pathogenesis of Alzheimer's disease. We have focused on 'membrane-mediated' amyloidogenesis because Yanagisawa et al. identified a specific form of Aβ that was bound to monosialoganglioside GM1 in brains exhibiting the early pathological changes associated with the disease. We have found that amyloid fibrils formed on GM1 clusters were more toxic than those formed in solution [1, 2]. The less toxic fibrils formed in solution are considered to be composed of in-resister parallel β-sheets, whereas the structure of the toxic fibrils is unknown, although FTIR spectra suggested the presence of antiparallel β-sheets [1, 2].
In this study, we investigated the structure of the toxic fibrils in detail. Solid-state NMR measurements using site-specifically [15N, 1-13C]-labeled Aβs suggested that the fibrils contained both parallel and antiparallel β-sheet structures. Chemical cross-linking experiments using Cys-substituted Aβs also supported this conclusion. Thus, the toxic fibrils were found to possess a novel unique structure.
[1] Fukunaga, S. et al., Biochemistry 51, 8125 (2012).
[2] Matsuzaki, K., Acc. Chem. Res. 47, 2397 (2014).

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Structural Transformation of Amyloid Peptides Interacting with Lipid Membranes
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The inherent cytotoxicity of protein aggregates implies a common mechanism for amyloid diseases (Bucciantini et al). However, accumulated evidence suggests that the insoluble fibrils or aggregates are not the culprit. On the other hand, amyloid peptides in soluble form do interact with lipid bilayers, suggesting that the cell plasma membranes are a target of amyloid pathogeneses. In particular, Keyed et al. have demonstrated that amyloid peptides all increase membrane ion conductance without any evidence of discrete channel or pore formation. In this study we try to find the common molecular process of soluble amyloid peptides interacting with lipid membranes that might induce membrane conductivity. It is difficult to study this molecular process for most amyloid peptides because of its propensity to fibrillize at relatively low solution concentration. PrP 106-126 is a random coil in its soluble form. We study its kinetics of structural transformation in the presence of lipid vesicles. The time dependence of the structural changes was analyzed as a function of the lipid concentration. We demonstrate that the soluble peptides transform from random coils to alpha-helices upon binding to lipid bilayers. The helical state is stable, as long as the bound peptide to lipid ratio P/L on the lipid vesicle is below a critical value P/L*. But as P/L exceeds P/L*, the peptides transform from the helical state to beta-aggregates. This is consistent with previous studies of penetration and hIAPP in multiple lipid bilayers. Thus we found the common fibrillization process of amyloid peptides interacting with lipid bilayers. Our proposal is that the process of peptides' transformation from random coils to helices and then to beta-aggregation creates defects in the membranes that allow ion permeation to occur as observed by Keyed et al.

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Alpha-Synuclein Stabilizes Small Unilamellar Vesicles by Reducing Both Membrane Surface Tension and Rigidity
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Alpha-Synuclein (aSyn) stabilizes small unilamellar vesicles (SUV) by reducing the vesicle's surface tension and membrane rigidity. Using coarse-grained molecular dynamics simulations we explored the membrane remodeling characteristics of aSyn bound to DPPC lipid vesicles. By combining our recently developed Spherical Harmonics fluctuation analysis with 3-dimensional pressure tensor calculations we were able to characterize aSyn's effect on both structure and mechanical properties of DPPC vesicles. Our findings highlight a dramatic reduction in membrane surface tension and increased membrane fluctuations, suggesting a less rigid bilayer, for the aSyn vesicle system relative to pure DPPC.

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Membranes can Finely Tune Peptide-Induced Lipid Extraction by Modulating their Lipid Composition
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Membrane binding of lytic peptides, and the resulting peptide-induced lipid extraction (membrane solubilization) are influenced by the nature of lipids forming the membranes. However, the impact of various lipid species on these two phenomena cannot be directly inferred one from the other; distinct peptide/lipid interactions are pivotal in these distinct events.
Melittin is a small cationic peptide with a secondary amphipathic helical character. It interacts spontaneously with lipid bilayers, and induces lipid extraction. We investigated how phosphatidylserine (PS), cholesterol, and phosphatidylethanolamine (PE) modulate melittin binding to phosphatidylcholine (PC) membranes as well as their impact on lipid extraction.
First, PS, a negatively charged phospholipids, increases melittin affinity for membranes but inhibits melittin-induced lipid efflux. It is proposed that the attractive electrostatic interactions between melittin and PS, which are responsible for the increased affinity, anchor melittin at the bilayer interface and prevent its relocation required for lipid extraction. Second, cholesterol reduces melittin membrane affinity. In parallel, it inhibits the extent of lipid extraction induced by melittin and LC-MS analysis indicates that lipid/melittin particles resulting from the extraction are cholesterol- depleted relative to the cholesterol content of the original membranes. It is proposed that the phospholipid ordering caused by cholesterol is unfavorable to peptide binding and inhibits the overall melittin action. Third, PE decreases the affinity of melittin for PC-based bilayers, an inhibition more significant in the gel than in the fluid phase. The limited amount of melittin that can be accommodated in PE-containing gel-phase bilayers appears to make the bilayers more susceptible to lipid extraction. As a consequence, PE can act as a promotor or an inhibitor of melittin-induced lipid extraction, depending on its proportion. These findings demonstrate that membranes can tune peptide-induced lipid extraction by altering their lipid composition.

2775-Pos Board B205
Mechanism of Action of β-Hairpin Antimicrobial Peptides
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Previous work showed that the β-hairpin antimicrobial peptide (AMP) protegrin forms stable octameric β-barrels and tetrameric arcs (half barrels) in both implicit and explicit membranes. Here, we extend this investigation to several AMPs of similar structure: tachyplesin, androctonin, polyphemusin, gomesin, and the retrocyclin θ-defensin. We also examine synthetic β-sheet peptides selected from a combinatorial library for their ability or inability to form pores in lipid membranes. When heptameric, octameric, and decameric β-barrels and tetrameric arcs of these peptides were initially embedded in preformed neutral and anionic lipid pores, a variety of behaviors and membrane binding energies were observed. The synthetic peptides bound very strongly and formed stable barrels and arcs in both neutral and anionic pores. The natural AMPs exhibited unfavorable or marginally favorable binding energy and kinetic stability in neutral pores, consistent with the lower hemolytic activity of some of them compared with protegrin. Binding to anionic pores was more favorable, but significant distortions of the barrel or arc structures were sometimes noted. These results are discussed in light of the available experimental data. The diversity of behaviors obtained makes it unlikely that the barrel and arc mechanisms are valid for the entire family of β-hairpin AMPs.

2776-Pos Board B206
Attack on Single Escherichia Coli Spheroplasts by Antimicrobial Peptides
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Studies of the molecular mechanisms of AMPs are mostly performed with lipid bilayers.Thus there is a persistent question as to whether the action of AMPs on bacterial membranes can be reproduced on lipid bilayers. Recently Weisshaar and coworkers have studied the actions of AMPs on *E. coli* and *Bacillus subtilis* by time-lapse fluorescence microscopy. The direct observation of the action of AMPs on bacteria revealed two key steps. The first is growth halting due to direct interference of AMP with cell wall synthesis and is recoverable. The second is permeabilization of the cytoplasmic membrane which is not recoverable. Here we study the direct action of AMPs on the cytoplasmic membranes by using E. coli spheroplasts, the cell form from which the outer membranes have been removed. The purpose is to compare the action on bacterial membranes with that on lipid bilayers. The key question is how to reveal the mechanisms of AMPs on bacterial membranes. First we observe the action of AMPs on giant unilamellar vesicles (GUVs) made of E. coli total lipid extract.We used the aspiration method to hold the GUV in a solution containing a soluble dye